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MITOSIS IN THE ROOT-TIP CELLS OF *PODOPHYLLUM PELTATUM.*

BY A. RICHARDS.

Plates XV and XVI.

(Contribution from the Botanical Laboratory of the University of Kansas.)

THE work, the results of which are herein presented, was undertaken during the summer of 1907, under the direction of Prof. W. C. Stevens, whom I have to thank for his kindness and much helpful advice. My thanks are also due Mr. F. A. Hartman for helpful suggestions.

In discussing the root-tip cells of *Podophyllum peltatum,* I shall use the following outline:

I. Methods.
II. Resting cells.
  A. Nucleus.
    1. Nuclear membrane.
    2. Chromatin.
    3. Achromatin.
    4. Nucleolus.
  B. Cytosome.
    1. Cell wall.
    2. Cytoplasm.
    3. Archoplasm.
III. Dividing cells.
  A. Prophase.
    1. Nucleus.
    2. Cytosome.
  B. Later phases of division.
    1. Chromatin.
    2. Achromatin and cytoplasm.
IV. General considerations.
  A. Achromatic and archoplasmic structures.
  B. Individuality of chromosomes.

METHODS.

The root-tips of *Podophyllum peltatum* are peculiarly fitted for the study of mitosis in the vegetative cells of plants. The cells are larger than those of the onion root-tip, and in properly stained material the cell organs, especially the chromosomes, are to be seen with almost diagrammatic clearness.

The material used in this study was collected during the latter part of March and fixed in Flemming. For staining the sections three methods were used: Haidenhain's iron-hæmatoxylin (some sections counterstained with orange G), the safranin-gentian violet-orange G stain, and a combination of these two. Satisfactory results were obtained with slides stained in hæmatoxylin, both with and without the counterstain; the three-color method also gave fair results. Only a few sections were successful with the combination method, but they were promising; without doubt the method will repay careful experiment. It was followed as given by Schaffner (2).

THE RESTING CELL.

The most typical resting cells are not often to be found at the tip of the root, for this, of course, is the region of greatest mitotic activity and the resting stages are very short, but back from the tip as much as two millimeters. Farther from the tip the cells are apt to be plasmolyzed in fixing.

The nuclear membrane of the resting cell normally disappears as soon as the cell begins division; it is, however, quite constant in its appearance and staining reaction, and varies only slightly in thickness. It appears to stain very deeply with chromatin stains, a fact which is probably due to the peripheral arrangement of the chromatin granules, as seen in figure 1. These chromatin masses are arranged over the linin network of the nucleus in large or small patches, depending on the age of the cell, *i. e.*, nearness to the tip.

In examining the achromatic structures of the cells of *P. peltatum* I have had considerable difficulty, especially in the stages of mitosis. In some cells they can be seen quite clearly; in others, which appear to be equally well fixed and have been treated with exactly the same methods, they cannot be seen at all. Owing to this irregularity in their staining capacity, I have not been able to work out their history accurately.

The nucleoli of these cells are very interesting. They vary

in number from one to several—rarely more than four—and in size and shape. In general they are irregular-spherical, but may be compressed into different shapes. The variation in size, which is considerable, depends on the age of the cell. As the time for division approaches they grow smaller, finally disappearing about the same time as the nuclear membrane. This last fact sustains the conclusion that the nucleolus is simply a storehouse for food material for the rest of the cell.

In most cells up to the time of division there is noticeable a peculiar vacuolated condition of the nucleus, and in these vacuoles lie the nucleoli. Figure 1 shows two such cells, one of which has a single large nucleolus surrounded by a vacuole and the other three. The term "vacuole" for the clear space surrounding the nucleoli may be misleading. Experiments seem to show that this colorless space contains something more than a watery fluid, which is extracted by dehydration. By subjecting the roots of *Zea mays, Vicia faba,* and other plants to strong centrifugal force, Mottier (1) found that the nucleolus with its surrounding clear space was thrown out of the nucleus into the cytoplasm. "The nucleolus was still surrounded by the space, a fact that seems to show that the colorless substance has greater specific gravity than the other contents of the nucleus. The colorless substance may represent the unorganized nucleolar material."

The cell wall of the resting cells stains much like the nuclear membrane and is only slightly thicker.

The cytoplasm stains fairly dark with iron hæmatoxylin, and shows its fine reticular structure quite plainly. Scattered uniformly over the threads of the reticulum are large granules. The cytoplasm of the cell stains slightly denser than the nuclear sap.

With regard to archoplasmic structures, not the slightest evidence was seen to indicate that such structures as have been described by Schaffner are ever present: *i. e.*, centrosomes and centrospheres.

DIVIDING CELLS.

The plane of division in these cells is mostly at right angles to the axis of the root. This rule, however, is by no means invariable, for numerous cases have been found with the division planes parallel to, or at an angle of forty-five degrees with, the longitudinal axis (figs. 14, 20).

When the nucleus begins division the chromatin masses arrange themselves in a spireme (figs. 2, 3) and the linin reticulum can no longer be seen. Perhaps the network has drawn tighter, the threads stretching out to form a framework for the spireme. This stage has been called "close mother skein" (Schaffner). In figure 2 the nucleoli are still present, but they are now without the surrounding clear spaces.

The spireme soon becomes looped with the heads pointing toward the poles. I have not observed any connection between this fact and the fact that the chromosomes are J- and U-shaped. The segmentation of the spireme does not seem to occur regularly near the equator of the nucleus, as would be necessary to make the heads of the chromosome loops identical with those of the spireme loops.

At about this stage the nuclear membrane disappears, as do the nucleoli. There is, of course, variation about the time of disappearance, for the membrane is still present in figure 4, where the granules have split to form a double spireme. This split is very clearly shown in figures 5 and 6. The spireme granules here have segmented to form the chromosomes. Immediately after segmentation the chromosome granules condense and the segments shorten, thus giving the apparently solid character to the chromosomes. In figure 6 the two lower segments have lagged behind the others in condensation.

At this stage or earlier the *anlage* of the spindle should appear, for in the next stage observed the chromosomes were arranged on the bipolar spindle in the metaphase. The origin of the spindle was not observed, however, nor were any traces of a multipolar spindle seen. This may be due to any one of the three causes: the duration of the multipolar spindle may be short; the spindle substance at this stage may not take the stain readily; or the bipolar spindle may have been formed in the manner usual in the cases of higher plants, but with the omission of the multipolar stage. Of these three hypotheses for the absence of the multipolar stage, the third seems, perhaps, the best from the evidence in hand. The probability of the hypothesis decreases when we consider that the central spindle fibers stain more deeply than the traction fibers. No centrosome or centrosphere was seen in these cells, although in one or two cases a massing of protoplasmic granules at the poles of the cells might have been taken on a superficial examination for a centrosphere (figs. 7, 11). It was not difficult to de-

termine, however, that such was not the case, and it seems quite certain that the spindle is formed without the influence of other archoplasmic structures.

In most cells there is no metaphase for the entire chromosome mass, *i. e.*, an equatorial plate, in these cells, but each chromosome passes through the stage without waiting for the others. Figures 7 and 11 are the only approach to such a condition, and even here there is some lack of uniformity in the division of the chromosomes. Figures 8, 9 and 10 show the usual condition of the equatorial plate. Figure 10 shows the longitudinal split of the undivided chromosomes very clearly. This cell was crushed, but the crushing served to bring out the split all the better.

During the metaphase the first signs are seen of the concentration of the cytoplasm at the center of the cell, where the new wall is to be formed, and the consequent vacuolation of the cytoplasm at the poles. (This phenomenon is the usual occurrence in these cells but there are numerous instances where it is only slightly marked.) This concentration seems to aid in the construction of the new cell plate, for the more marked the concentration the more rapid the cell-plate formation.

In regard to the division of the chromosomes nothing unusual was observed. The chromosomes divide and pass to the poles as in most forms. In passing up to the poles the chromosomes usually turn with the short arm of the J toward the center of the spindle. One chromosome seemed to stand out from the rest slightly and was especially marked by its size; this was the long chromosome *m* (figs. 9, 14). Polar views of the anaphase present the best opportunities for counting the chromosomes, but there is much uncertainty even here, owing to the difficulty of getting all of the plate in one section or of tracing it through neighboring sections. Fourteen chromosomes were counted in about a dozen cells, but this, of course, is insufficient evidence on which to base a decision.

Arrived at the poles the chromosomes fuse end to end and then form a loose daughter "skein." This "skein" becomes a close one and the chromatin forms a dense mass at the poles. The remainder of the nuclear reconstruction consists in the formation of the membrane, the distribution of the chromatin granules in the nuclei, the appearance of the linin network and the formation of new nucleoli. It is a general rule that the nucleoli arise on the side of the cell nearest the new cell wall.

While the reconstruction of the nuclei has been in progress, several changes have occurred in the spindle and the cytoplasm. As soon as the concentration of the cytoplasm is complete the spindle broadens out somewhat. Figure 23 shows a maximum bulging, while figure 22 is perhaps a minimum. Figure 22 shows an unusual phenomenon in the process; the majority of the central spindle fibers (only the central fibers now remain) seem to have become free at their polar ends and to have shortened towards the center, staining very darkly in this region. The threads disappear as soon as the cell walls are formed. It is difficult to determine what the bulging out of the spindle and the concentration of the threads and cytoplasm signify, but it seems probable that the threads contain some substance useful in the construction of the new cell wall. This suggestion is in line with the work of Strasburger (4) and Haberlandt (5), who have discussed this phenomenon of the spindle in detail.

The rate at which these processes occur varies. One may find a nuclear membrane before the cell plate has started (fig. 20), or he may find the cell plate well started while the chromatin is still in the loose daughter "skein" (fig. 19).

GENERAL CONSIDERATIONS.

Achromatin and Archoplasm.—In regard to the origin of the spindle Schaffner concludes that, in root-tips of the onion and of *Sagittaria*, at least, the multipolar spindle is due either to pathological causes or to "improper manipulation in preparing the sections," and that a bipolar spindle is formed not by the transformation of a multipolar stage but under the influence of a centrosome and centrosphere as commonly described for animals. As previously stated, I find no evidence whatever for concluding that such archoplasmic structures are present.

Although no traces of a multipolar spindle have been seen here, it is not necessary to assume that a centrosome is present to guide the formation of the bipolar spindle, as the fibers themselves may possess sufficient directive energy to bring about the bipolar form.

Individuality.—A single bit of evidence was found in support of the theory of individuality of the chromosomes in vegetative cells. In several of the cells there is an unusually long chromosome, *m*, in general dividing before the others in the cell. Although this meager bit of evidence is by no means conclusive,

it points toward the view that while the cell is the unit of structure the chromosome is the unit of potentiality, and that the chromosomes themselves may all differ in structure and, if in structure, also in potentiality.

An interesting problem concerning these root-tip cells is the relation of the cells to the entire tissue and the cytological processes of later stages of embryonic growth. Material for this study is not at hand at this time, but I hope to be able to trace out these processes at some subsequent period.

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NOTE.

The drawings of the *Podophyllum* root-tips were all made with the aid of a camera lucida (B. & L.) A C. Reichert 1/12 objective was used and with it a B. & L. ½-inch ocular for the larger figures and a ¾-inch for the smaller, making a magnification of 2466 and 2000 diameters, respectively.

Figures 9, 13, 14, 15, 16, 18, 20, 22, 23, 24, were made from material stained with the Flemming three-color method. The others were made from sections stained with the iron-hæmatoxylin method.